

CLAIMS

What is claimed is:

1. A method of producing a host cell capable of producing a product of interest, comprising:

transfecting a host cell culture with a DNA construct comprising a transcriptional regulatory region, a fused selectable gene sequence and a gene encoding a product of interest;

directly culturing the transfected host cells in a selective medium;

allowing the host cells to grow in the selective medium for a sufficient time to allow amplification of gene encoding the product of interest to occur; and

selecting a host cell clone that is capable of producing at least about 250mg/l of the product of interest.

2. A method of claim 1 wherein the selective medium contains at least about 25nM methotrexate.

3. A method of claim 1 wherein the selective medium contains at least about 50nM methotrexate.

4. A method of claim 1 wherein the host cell is a CHO cell.

5. A method of claim 1 wherein the product of interest is a protein selected from the group consisting of an antibody, enzyme, hormone, lipoprotein, clotting factor, anti-clotting factor, cytokine, viral antigen, chimeric protein, transport protein, regulatory protein, homing receptor, and addressin; or a fragment of said protein.

6. A method of claim 1 wherein said product of interest is a humanized antibody.

7. A host cell produced according to the method of claim 1.

8. A method of producing a product of interest, comprising culturing a host cell produced according to the method of claim 1 under conditions suitable to cause expression of the product of interest in an amount at least about 250mg/l.

9. A method of claim 1 wherein the DNA construct comprises, in order 5' to 3':

a) a transcriptional regulatory region capable of regulating transcription of both the selectable gene and the product gene;

b) a transcriptional initiation site;

c) a fused selectable gene sequence positioned within an intron defined by a 5' splice donor site comprising a splice donor sequence such that the efficiency of splicing messenger RNA having said splice donor sequence is between about 80% and 99% as determined by PCR, and a 3' splice acceptor site;

d) a product gene encoding a product of interest; and

e) a transcriptional termination site.

10. The method of claim 9 further comprising recovering the product of interest from the culture.

11. A method of claim 9 wherein the transcriptional regulatory region capable of regulating transcription of both the selectable gene and the product gene is driven by a SV40 promoter.

12. A method of claim 9 wherein the transcriptional regulatory region capable of regulating transcription of both the selectable gene and the product gene is driven by a CMV promoter.

13. A cell culture composition comprising a host cell according to claim 9 and at least about 250mg/l of the product of interest.

14. A method of producing a host cell capable of producing at least about 250mg/ml of a product of interest comprising transfecting a host cell with a DNA construct comprising in order from 5' to 3':

a) a transcriptional regulatory region capable of regulating transcription of both the selectable gene and the product gene;

b) a transcriptional initiation site;

c) a fused selectable gene sequence positioned within an intron defined by a 5' splice donor site comprising a splice donor sequence such that the efficiency of splicing messenger RNA having said splice donor sequence is between about 80% and 99% as determined by PCR, and a 3' splice acceptor cite;

d) a product gene encoding a product of interest; and

e) a transcriptional termination site;

wherein the transfection is performed in suspension culture.

15. A method of claim 14, wherein the DNA construct is introduced into the host cells by lipofection.

16. A method of claim 14 wherein said transfection is performed in a spinner vessel.

17. The method of claim 14 wherein the suspension culture has cell density of at least about 5×10^5 /ml at the time of transfection.

18. The method of claim 14 wherein the suspension culture has a cell density of at least about 1.5×10^5 /ml at the time of transfection

19. A method of claim 15 wherein the product of interest is selected from the group consisting of an antibody, enzyme, hormone, lipoprotein, clotting factor, anti-clotting factor, cytokine, viral antigen, chimeric protein, transport protein, regulatory protein, homing receptor, and addressin and a fragment of any of said product of interest.

20. A method of rapidly selecting a host cell producing a product of interest, comprising:

transfecting a host cell culture with a DNA construct comprising a transcriptional regulatory region, a fused selectable gene sequence and a gene encoding a product of interest;

directly culturing the transfected host cells in a selective medium; and

allowing the host cells to grow in the selective medium for a sufficient time to allow amplification of gene encoding the product of interest to occur.